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Unpacking, Positioning and Installation

- Inspect the instrument for any signs of damage caused in transit. If any damage, inform your supplier immediately.

- Ensure your proposed installation site conforms to the environmental conditions for safe operation :

  Indoor use only
  Temperature 10°C to 40°C
  Maximum relative humidity 80 % up to 31°C decreasing linearly to 50% at 40°C

- The instrument must be placed on a hard flat surface, for example a laboratory bench or table, which can take the instrument weight (13 kg) such that air is allowed to circulate freely around the instrument.

- Ensure that the cooling fan inlets and outlets are not obstructed; position at least 2 inches from the wall.

- This equipment must be connected to the power supply with the power cord supplied and MUST BE EARTHED (GROUNDED). It can be used on 100-240V supplies. The instrument rear panel is shown on the inner back cover of this manual.

- Switch on the instrument and check that the display works (see OPERATION).

- The instrument is delivered with a stored baseline. This is required to correct for the wavelength/ energy profile of the light source. A new baseline should be stored when a lamp is changed or if the instrument is not used for a long time (several weeks); refer to Maintenance for details.

- To enter laboratory name, operator name and/or instrument asset number details at this stage, refer to Appendix, Entry of alphanumeric characters for print out.

If this equipment is used in a manner not specified or in environmental conditions not appropriate for safe operation, the protection provided by the equipment may be impaired and instrument warranty withdrawn.

Essential Safety Notes

There are a number of warning labels and symbols on your instrument. These are there to inform you where potential danger exists or particular caution is required. Before commencing installation, please take time to familiarise yourself with these symbols and their meaning:

Caution (refer to accompanying documents).
Background colour yellow, symbol and outline black.

Accessories - Care should be taken when handling all heated accessories.

- Ensure that the cell compartment lid is closed when operating cell changers and the sipper.

- It is essential that the baseplate plug supplied with single cell accessories is fitted to optimise air flow and prevent light ingress.

Symbols used in Manual

Please Note

Warning

Insert cell containing reference into cell holder.

Display Panel

Key Press

Key Press

Abs

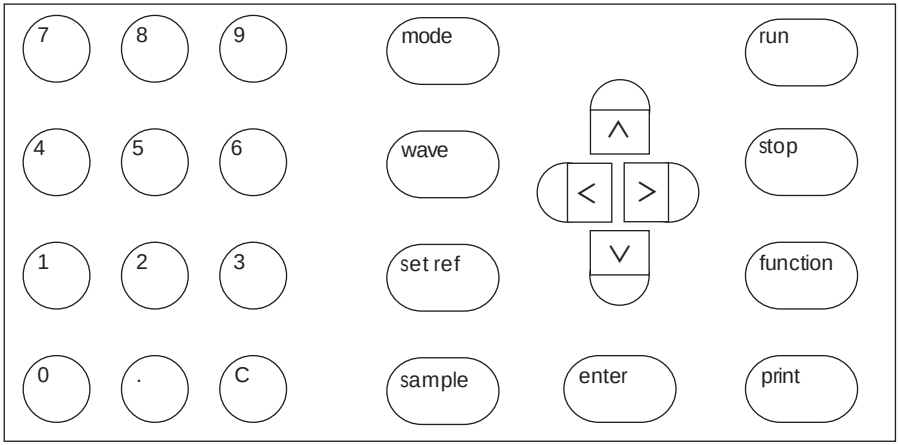
Absorbance

λ

Wavelength

Insert cell containing sample into cell holder.

Keyboard



Ignoring the numeric keypad, the keyboard is designed to be operated from top to bottom and from left to right. Basic absorbance measurements are very easy to make. In addition, a range of instrument utilities and instrument modes is accessible when the **function** or **mode** key is pressed; these options are described later.

(mode) Select measurement mode required using [<] [>] [∧] [∨] keys. Press (enter)

(wave) Select wavelength either from the numeric keypad or by using [<] [>] keys. Press (enter)

(set ref) Set absorbance and transmittance readouts to 0.000 absorbance units and 100 % T, respectively, on a reference solution.

(sample) Select sample position in cell changer either from the numeric keypad or by using [<] [>] keys. Press (enter)

(enter) Select options on the display. (C) Clears a numeric entry.

(run) Start measurements when operating from within the measurement modes.

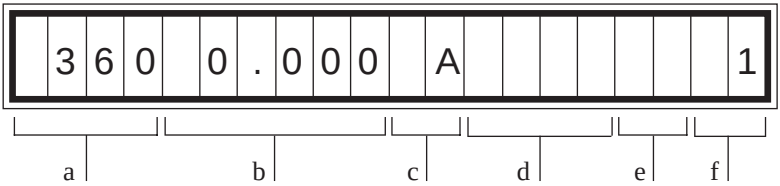
(stop) Ends current programme.

(function) Access to Set-up and Parameters.

(print) Output information on the display panel to a parallel printer.

Display Panel

The display panel has 20 characters divided up as indicated. Default start-up values are shown, and represent a wavelength of 360nm in absorbance mode, with cell position number 1 in the light path.



a) **Wavelength**
Display of current wavelength in nm.

b) **Numeric Result**
Display of absorbance, % T, concentration, absorbance ratio result.

c) **Unit of Measurement**
Display of numeric result unit (**A** is Absorbance Units; **%T** is % Transmission; **C** is concentration units).

d) **Mode of Operation**
Display of instrument enhancement mode :

FC and **SC** are factor and standard concentration modes;
DNA, RNA, OLI are DNA, RNA, oligonucleotide quantification modes;
PRO is protein concentration mode;
RAT is absorbance ratio mode;
KNR is display of absorbance with time;
KND is display of change of absorbance with time;
SCN is scan to chart recorder.

e) **Temperature Indicator**
Display of temperature status if a peltier heating accessory is in use.

ºC is at temperature;
HI is over temperature or cooling down;
LO is under temperature or heating up.

f) **Sample Number**
Display of sample number.

OPERATION

Introduction

Your UV/Visible spectrophotometer is a simple to use, microprocessor controlled instrument.

It works on the basis of light from either of the lamp sources being directed by a motorised mirror through the monochromator inlet slit. This passes through one of several (dependent on wavelength selected) filters mounted on a filter quadrant; the filtered light is then directed onto the holographic grating which produces light of the selected wavelength. The light then leaves the monochromator via the exit slit, and mirrors focus and direct the light into the sample compartment. This passes through your cell containing the sample of interest and a defocussing lens to a solid state detector unit. The resulting signal is then amplified and displayed.

Your spectrophotometer :

- measures standard absorbance, concentration and transmittance.
- has stored parameters for DNA, RNA and oligonucleotide quantification and purity checking, as well as for protein contamination measurement in nucleic acid solutions.
- measures absorbance ratios.
- measures change of absorbance with time and can either print the numerical data or output the absorbance time plot to a chart recorder (synchronised).
- outputs a wavelength scan to a chart recorder (asynchronous).
- can be connected to a standard centronics parallel printer for output of results.
- can be linked to a PC via its serial interface and used with the SWIFT range of software application programs operating in the Windows environment.

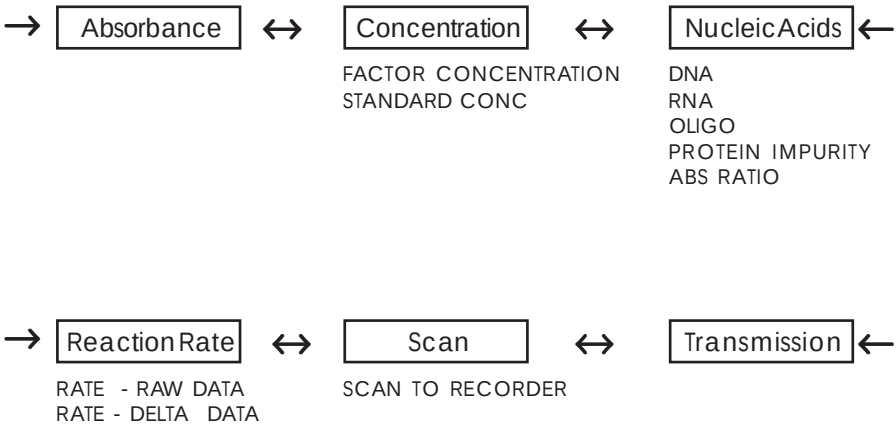
A range of accessories further enhances the capability of the instrument.

Measurement Modes

mode

The mode key gives access to the 6 operational modes; these are linked in a continuous loop. After pressing mode, use the arrow keys to move up/down/left/ right in the matrix shown below. Each mode is described subsequently.

Select from the table below with mode < > ^ v enter



mode < > ^ v enter to choose required measurement mode.

By pressing the mode button within a measurement mode the user can return to the previous step of operation.

The instrument can be switched off at any stage of operation.
A flashing cursor indicates an option which is currently selected.

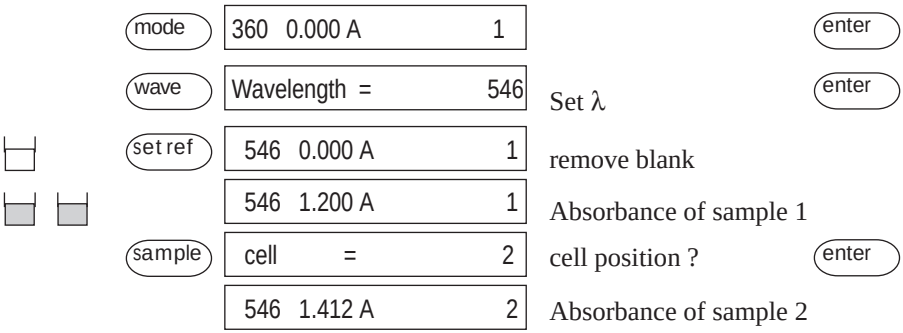
Absorbance

mode

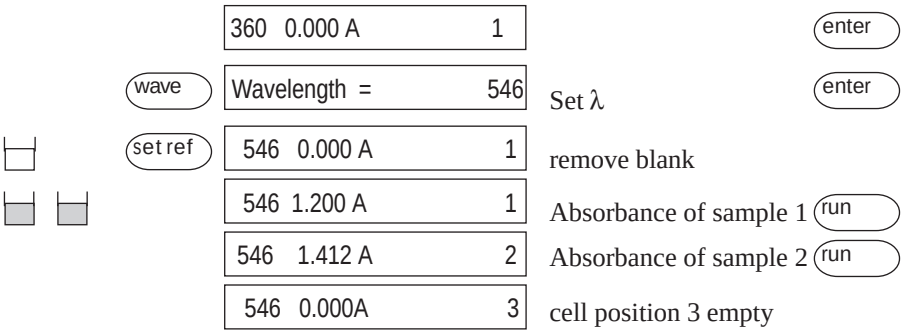
Absorbance mode is the default after power on. It is used to perform simple absorbance measurements on samples, and it measures the amount of light that has been passed through a sample relative to a blank (this can be air).

The procedure is to select the appropriate wavelength, insert blank and set reference on it, remove the blank, and insert the sample(s). If **sample** is pressed and cell position 2 selected, the display will show the wavelength used and the absorbance and cell position of the sample in the light path. If **run** is pressed the sample absorbance is measured and the cell changer rotated sequentially to the next position; the display therefore shows the wavelength used, and the absorbance and cell position of the next sample, since it is now in the light path. The advantage of using run is that if a printer is connected and RUN PRINT in Setup selected, then there is automatic print out of the absorbance details of the sample just measured.

Using (sample) with 2 samples



Using (run) with 2 samples



set ref — to set absorbance to 0.000 AU on a reference solution at all wavelengths in the mode selected. During a standard operating procedure, the user is prompted to insert a cell containing reference into cell holder, by the message set reference. This is displayed until the reference is inserted and set ref is pressed.

run — to start making measurements; sample number (and cell position) are automatically incremented after measurement.

stop — to stop making measurements or return to Absorbance mode.

sample > — increments sample number (and cell position if cell changer is fitted).

The above and following sequences of instructions apply to the 6 cell changer which is supplied with the instrument. There are different self explanatory displays if a single cell holder or sipper is installed.

and can be put in sequentially if a 6 cell changer is fitted.

Concentration

mode

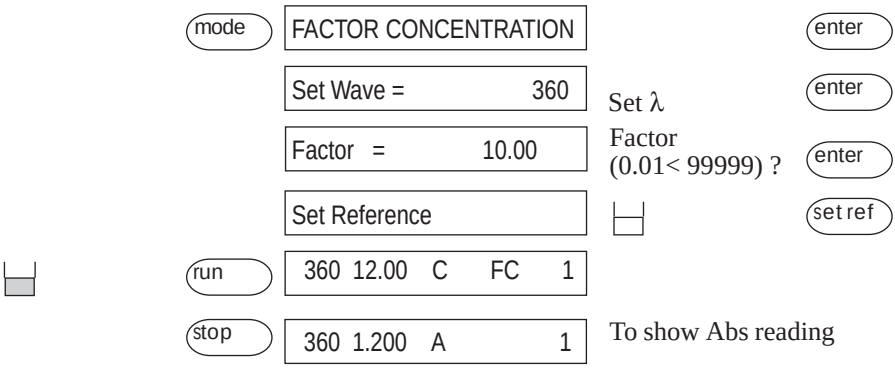
There are two concentration modes, Factor and Standard.

Factor Concentration mode is used when a conversion factor is known; this is required to convert the absorbance measurement for a sample at a specific wavelength to a concentration, by a simple multiplication of absorbance x factor.

Standard Concentration mode is used when a sample of known concentration is available; by measuring the absorbance of this at a specific wavelength, the conversion factor is calculated (see above), and this can be applied to other samples of unknown concentration. This is equivalent to a one point calibration, and assumes that a sample of zero concentration has zero absorbance.

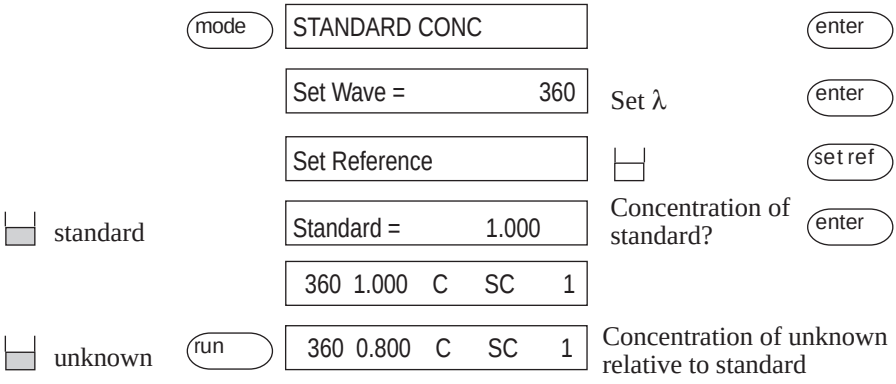
FACTOR CONCENTRATION

Concentration of unknown = Absorbance x factor



STANDARD CONC

Concentration of unknown = Absorbance of unknown x $\frac{\text{Concentration of standard}}{\text{Absorbance of standard}}$



The calculated factor cannot be displayed.

There are 5 modes for Nucleic Acid Quantification studies using absorbance measurements at 260 and 280nm.

Mode	Use
DNA	DNA quantification and purity checking
RNA	RNA quantification and purity checking
Oligo	Oligonucleotide quantification and purity checking
Protein Impurity	Estimation of protein concentration in nucleic acid solutions
Abs Ratio	For general use, and for DNA, RNA and oligonucleotides if a dilution factor is to be included

DNA RNA Oligo

Nucleic Acid Quantification

Concentration can be measured using absorbance at 260nm because it is well established that a solution of DNA or RNA with an optical density of 1.0 (1.0 Absorbance Unit) has a concentration of 50 or 40µg/ml, respectively, in a 10mm pathlength cell*. Oligonucleotides, as a rule of thumb, have a corresponding factor of 33µg/ml, although this does vary with base composition *. Use Factor concentration mode if you wish to use a different factor.

Concentration = $A_{\lambda 260}$ * Factor

Nucleic Acid Purity Checking

Absorbance ratio can be used to establish the presence of impurities in a sample preparation, relative to a pure sample. The two wavelengths of interest to the Molecular Biologist are the absorbance maxima of the nucleic acid, 260nm, and the protein impurity, 280nm. A deviation from the expected absorbance ratio of the two wavelengths for the pure substance indicates the presence of impurity in the sample:

Absorbance ratio = $A_{\lambda 260} / A_{\lambda 280}$

The $A_{\lambda 260} / A_{\lambda 280}$ absorbance ratio is known for the pure nucleotide (1.8 for pure DNA, 2.0 for pure RNA), enabling rapid assessment of quality.

Background correction

A wavelength which is totally separate from these peaks, 320nm is sometimes used to compensate for the effects of background absorbance due to, for example, turbidity or high absorbance buffer solution.

Concentration = $(A_{\lambda 260} / A_{\lambda 320})$ * Factor

Abs ratio = $(A_{\lambda 260} - A_{\lambda 320}) / (A_{\lambda 280} - A_{\lambda 320})$

If your laboratory has not used background corrections before, set this option to NO.

* Reference : Molecular Cloning, Maniatis et al.

Summary

Nucleic Acid sample type	Factor, µg/ml	$A_{\lambda 260} / A_{\lambda 280}$
ds DNA	50	1.8
RNA	40	2.0
Oligonucleotide	33	sequence dependent

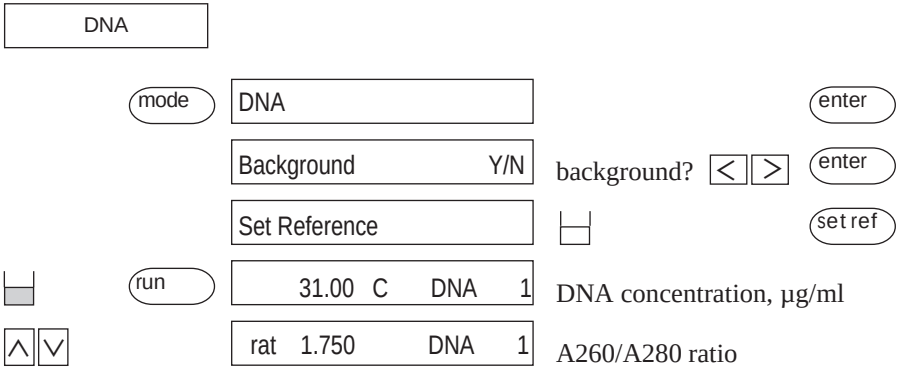
Notes :

If you wish to use other factors, for example by incorporation of a dilution factor, or other wavelengths, for example if the peak maximum is at 257nm or a background wavelength at 350 nm, use Absorbance Ratio mode (ABS RATIO).

When using these modes with restricted aperture cells, for example the 7µl ultramicrovolume cell, ensure that they are correctly filled by holding up to the light. This is to avoid the possibility of beam clipping, and ensures that the results obtained are reproducible.

Background compensation at 320nm is used to correct for high absorbance buffer solution. It is also helpful if using low absorbance solutions in ultramicrovolume cells.

If a printer is connected, the results, including absorbance values at 260, 280 and 320 nm (if selected), are printed out automatically. If a printer is not connected, the absorbance values are displayed for a few seconds in turn before showing the concentration and ratio results.



RNA

mode | RNA | enter

Background Y/N | background? < > | enter

Set Reference | set ref

run | 28.00 C RNA 1 | RNA concentration, µg/ml

rat 1.900 RNA 1 | A260/A280 ratio

OLIGO

mode | OLIGO | enter

Factor = 33.000 | Factor? | enter

Background Y/N | background? < > | enter

Set Reference | set ref

run | 15.00 C OLI 1 | Oligo concentration, µg/ml

rat 1.600 OLI 1 | A260/A280 ratio

DNA

USING A SINGLE CELL HOLDER ACCESSORY
A similar format is used for RNA and Oligo

mode | DNA | enter

Insert Reference | enter

Background Y/N | enter

Set Reference | set ref

run | 31.00 C DNA 1 | DNA concentration, µg/ml

rat 1.750 DNA 1 | A260/A280 ratio

Protein Concentration in Nucleic Acids

For estimation of protein concentration in Nucleic Acid solutions, one can automatically calculate the protein based on an equation derived by Warburg and Christian for crystalline yeast enolase*. This uses absorbances at 280 and 260 nm, and is valid provided tyrosine and phenylalanine are present:

Protein (mg/ml) = 1.55*(Abs $_{\lambda 280}$) - 0.76*(Abs $_{\lambda 260}$)

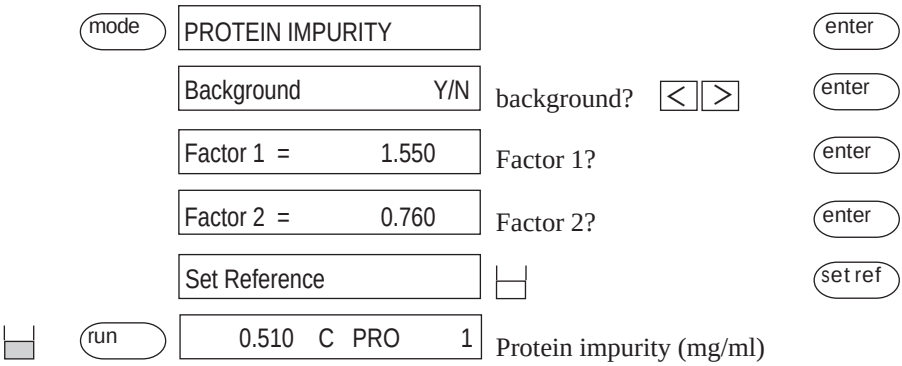
It is possible to calculate the coefficients in order to customise the equation for a particular protein, by measuring absorbances at known concentrations for both 260 and 280 nm; this generates a series of simultaneous equations which need to be solved for x and y (the required coefficients). In cases where x is calculated to have a negative coefficient, it should be set to zero; a negative coefficient arises because there is no contribution to the protein concentration due to the absorbance at 260 nm. The effects of background absorbance can be compensated for, if required.

* Reference : Warburg and Christian, Biochemisches Zeitung 310, 384 (1941).

Protein Conc =Factor 1 x (Abs $_{\lambda 280}$ - Abs$_{\lambda 320}$) - Factor 2 x (Abs $_{\lambda 260}$ - Abs $_{\lambda 320}$)

λ320 (background) is optional. Factors can be changed from defaults.

Set factor 2 = 0.00 for direct λ280 UV protein measurement. If BSA (bovine serum albumin) is an acceptable standard, setting factor 1 = 1.115 will give linear results from 0 to 0.8 mg/ml.

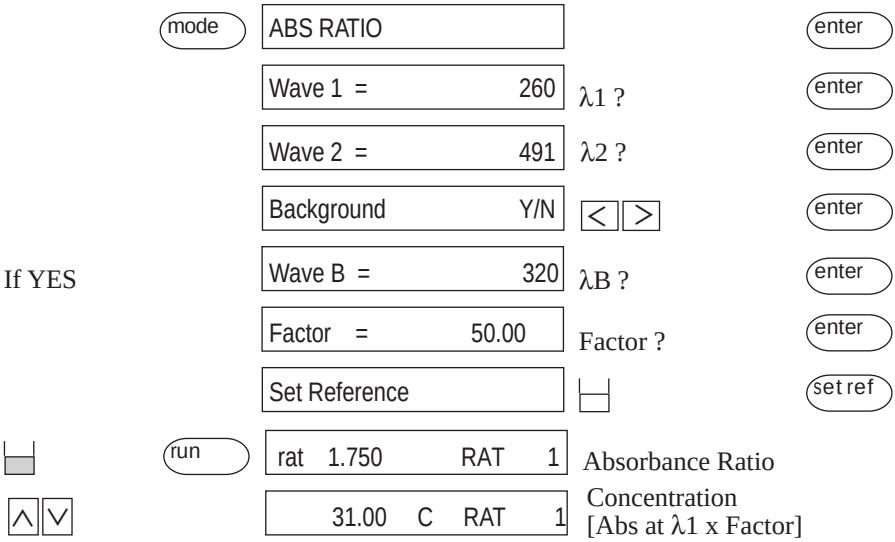


ABS RATIO

DNA, RNA, Oligo modes will give quantification results in µg/ml directly if a 10mm pathlength cell and no dilution by using the conversion factors of 50, 40 and 33 respectively. To get results directly using a 5mm pathlength cell and/or a solution which has had, for example, a 10 times dilution, the factor must be changed. To do this, use Abs Ratio mode and set wavelengths accordingly.

If using 5mm pathlength cell, for DNA quantification, use Factor = 100

If using a 10mm pathlength cell for DNA quantification and 10 x dilution, use Factor = 500



Reaction Rate

Rate studies can be used to measure the change in absorbance of a sample with time; a typical example is an enzyme kinetics reaction rate study at 340 nm. In this, the change in concentration of either one of the substrates or one of the products of the reaction is monitored at a particular wavelength. Another example is OD measurement in bacterial cell cultures at 600 nm.

Measurements such as absorbance (or concentration directly if an appropriate factor is applied) as a function of time, either in minutes or seconds, can be made with your instrument.

Delay time defines at what time into the assay the spectrophotometer starts to collect data, thereby accounting for a lag phase.

Time Interval defines the time between each absorbance measurement.

Duration is the total length of time, including delay time if applicable, for the assay.

The presentation of results can be either as actual absorbance (or concentration) at each defined time interval/period (RAW DATA) or as change of absorbance (or concentration) per unit time interval (DELTA DATA). When DELTA DATA results are constant, the reaction is proceeding at a linear rate.

RAW DATA results can be output to a chart recorder for a visual interpretation of the change with time; both RAW and DELTA DATA can be numerically output to a parallel printer.

Note that when in Rate Modes, data is sent as an ASCII stream to the serial interface - this can be picked up by a PC with Windows installed. Use the terminal emulator to pick up the datastream (settings are 19200 Baud, 1 stop bit, 8 data bits, 0 parity). After the reaction is finished, paste the results into Excel and parse into separate column using Data>Parse command.

RATE - RAW DATA display of actual absorbance with time (KNR).

mode	RATE - RAW DATA		enter
	Set Wave = 600	Set λ	enter
	Time unit sec/min	Seconds or Minutes? < >	enter
	Delay = 0	Delay Time (0≤999)?	enter
	Interval = 1	Time Interval (1≤99)?	enter
	Duration = 500	How much time including delay (5*interval≤10,000)?	enter
	Chart recorder Y/N	Use chart recorder? < >	enter
If YES	Chart post delay Y/N	Start chart recorder after delay?* < >	enter
	Chart off at end Y/N	Stop chart recorder after experiment?* < >	enter
If NO	Factor = 1.000	Factor?	enter
	Set reference Y/N	Set reference? If Yes < >	set ref
	600 0.375 A KNR 1		
run	600 0.365 A 0 : 03 : 18	Display of raw absorbance * factor updated every time interval.	

* Output to the Amersham Pharmacia Biotech REC 101 chart recorder is synchronised. Chart recorder cable (80-2105-95) is required. Suggested chart recorder settings are 10mm/second, 200 mV full scale deflection (output is 100 mV for 1.000 Abs unit), recorder on, zero suppress on zero, set to internal.

The instrument will "beep" at every time interval if "sound is ON". To change this, go to Set-up (See Instrument Utilities)

To abort an assay, press STOP.

It is not possible to perform parallel kinetics or multi samples, with this mode. To do this, use the SWIFT -KIN software module which is available for use on PC.

RATE - DELTA DATA display of change of absorbance with time (KND).

mode	RATE - DELTA DATA		enter
	Set Wave = 600	Set λ	enter
	Time unit sec/min	Seconds or Minutes? < >	enter
	Delay = 0	Delay Time (0≤999)?	enter
	Interval = 1	Time Interval (1≤ 99)?	enter
	Duration = 500	How much time including delay (5*interval ≤ 10,000)?	enter
If NO	Factor = 1.000	Factor?	enter
	Set reference ? Y/N	Set reference? If Yes ⌴ < >	set ref
	600 0.375 A KND 1		
⌴ run	600 -0.010 A 0 : 03 : 18	Display of absorbance difference * factor updated every time interval.	

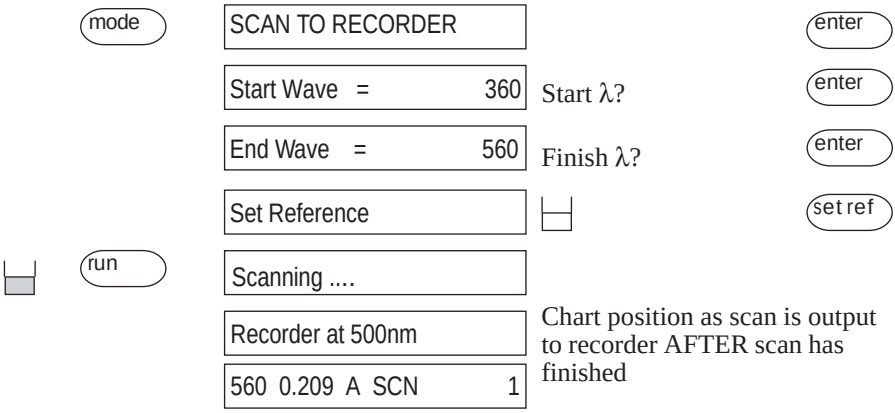
The instrument will "beep" at every time interval if "sound is ON". To change this, go to Set-up.

To abort an assay, press STOP.

Change in absorbance data can only be output to a parallel printer and not to a chart recorder.

Scan

An absorption spectrum can be obtained with your instrument by output to chart recorder. Ensure chart recorder is connected, as scan data is not visible without this. The Amersham Pharmacia Biotech REC 101 is recommended.

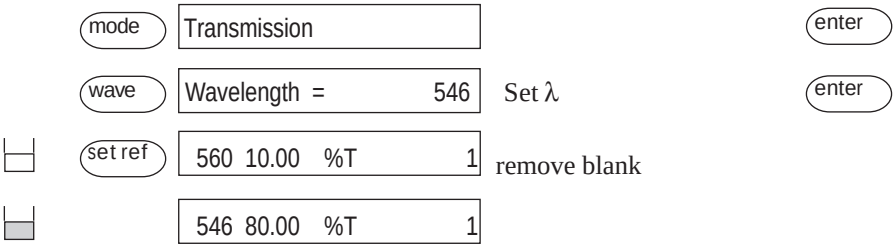


Output to the Amersham Pharmacia Biotech REC 101 chart recorder is asynchronous (the chart paper drive is controlled on/off automatically). Chart recorder cable (80-2105-95) is required. Suggested chart recorder settings are 10mm/second, 200 mV full scale deflection (output is 100 mV for 1.000 Abs unit), recorder on, zero suppress on zero, set to internal.

If you require post run data manipulation routines, the SWIFT-SCAN software module is available for use on PC.

Transmission

In transmittance mode the instrument measures the amount of light at the specified wavelength that has passed through the sample and compares it with that which has passed through the reference. This is displayed as a percentage. The relationship between the concentration of the sample and its transmittance at any given wavelength is not linear, and hence transmittance mode is rarely used experimentally except for samples having very high absorbances (low transmittances)



Instrument Utilities

Settings which can be changed are stored until overwritten.

Select from the table below with (function) < > ^ v (enter)

Select On/Off and Y/N Options with (enter) < > (enter)

Set a new DATE or TIME with (enter) < > keypad (enter)

→ Parameters ↔ Set-up ↔ Service ↔ Exit ←

Parameters	Set-up	Service	Exit
4090 V1.9 19960102	UV LAMP	This is reserved for Service Engineers	Return to absorbance mode
UV LAMP LIFE 27 H	NEW BASELINE		
VIS LAMP LIFE 27 H	SAVE BASELINE		
TOTAL USE 27 H	VIS LAMP		
BASELINE 19960102	UV LAMP SAVE		
DATE 19960102	SOUND		
TIME 11:16:32	RUN PRINT		
LAB NAME	GLP PRINT OUT		
OPERATOR	RE-CALIBRATE		
INSTRUMENT	LAMP CHANGE AT		
	LOCK/UNLOCK		
	RESET LAMP LIFE		

Dates are shown in year, month, day format

Parameters

Instrument parameters is a reflection of both the current instrument internal parameters (EPROM version; lamp lives; date of baseline) and the user definable documentation (time; date; laboratory; operator and instrument names); this latter part should normally require one time user access only. All parameters have battery back up.

4090 V1.9 19960102	EPROM version and date of software
UV LAMP LIFE 27 H	Indicates deuterium lamp usage in hours
VIS LAMP LIFE 27 H	Indicates tungsten lamp usage in hours
TOTAL USE 27 H	Indicates total instrument usage in hours
BASELINE 19960102	Indicates date last baseline was run (saved or not)
DATE 19960102	Set current date in year, month, day
TIME 11:16:32	Set current time in hours, minutes, seconds
LAB NAME OPERATOR INSTRUMENT }	These are printed out, if GLP PRINT OUT is set to Yes, for you to complete manually. Refer to Appendix if you wish to enter your details for printing out (page 41)

Set-up

Set up refers to instrument operating parameters, and include facilities for switching lamps off, running a baseline, setting the instrument to work in GLP mode, having automatic print on pressing RUN etc. These would normally be set up one at a time, on different occasions, and once changed, the instrument reverts to its default state (Absorbance measurement mode). Set up has battery back up.

UV LAMP
Switch deuterium lamp on or off.

NEW BASELINE
Create a temporary baseline to correct for the wavelength/energy profile of the spectrophotometers light source (s) (and reference if required). Ensure lamps are warmed up (30 minutes for deuterium). A temporary baseline is lost at instrument power down unless saved.

SAVE BASELINE
Save a temporary baseline to permanent memory. This can be the temporary baseline created above.

VIS LAMP
Switch tungsten lamp on or off.

UV LAMP SAVE
Cause instrument to power up and calibrate as usual, but then switch off the deuterium lamp automatically (infrequent users of the UV range can benefit from considerable increase in deuterium lamp life by use of this utility).

SOUND
Switch sound on or off when keyboard (and Sipper, if fitted) buttons are pressed, and at intervals in Rate modes.

RUN PRINT
Prints what is currently shown on the display, sending it to the printer every time RUN is pressed or when the display is updated (as in Reaction Rate).

GLP PRINT OUT
Print out a header at each calibration (or re-calibration) with information to conform with GLP if a printer is connected (setting GLP to OFF does not affect instrument calibration) - there is a 10 minute delay while the instrument warms up. Pressing **enter** after calibration confirms that the instrument status is acceptable. In addition, when **run** is pressed, the underlying readings and results are printed out.

RE-CALIBRATE
Causes instrument to re-calibrate (see GLP PRINT OUT).

LAMP CHANGE AT
Set the cross over wavelength at which instrument changes from UV (deuterium) to visible (tungsten) lamp; range is from 290 to 380 nm, default is 325 nm. Use only if there is critical detail at or around 325nm

LOCK/UNLOCK
Lock instrument into its current operating mode by entering a four digit number. When instrument is locked, you can only cycle between Absorbance, Locked Mode and % Transmission on using **MODE** [<] [>] keys.

Unlock instrument from its current operating mode by entering the four digit number used to lock it; if you forget the number, contact your local Technical Specialist.

RESETS LAMP LIFE
Resets UV and/or visible lamp life to 0 hours when a new lamp is fitted and creates new baseline. It also causes a reset of the maximum lamp energy, for GLP.

Service

Available for service engineer access only.

Exit

Returns instrument to normal operating condition, with new setup parameters in place.

Print Out

Your instrument will print out in 40 character format on any centronics compatible parallel printer connected via the appropriate cable to the printer port on the rear panel; simply press (print) to print current display.

a) **Print instrument display every time it is updated or a measurement is run:**

(function) > Set-up V RUN PRINT (enter) > on/off

Example (Absorbance Ratio DNA)

concentration

ratio

```
        31.00  C    DNA   1

rat     1.750       DNA   1
```

b) **Print a header at the start of an experiment :**

(mode) (print) for header print out on demand

Example (Absorbance Ratio DNA with GLP on)

```
Mode of use :-

DNA: concentration of DNA mode

DNA concentration = Abs 260nm * 50
   units are: ug DNA / ml
DNA ratio Abs at 260nm / Abs at 280nm

GLP calibrated 19960917 at 15:12
GLP Calibration PASS
Instrument status accepted

Operator  ................
Date  19960917    Time 12:03:50

                  260   0.620
                  280   0.354
      31.00 C     DNA  2
rat    1.750      DNA  2

cell changer reset to cell 3
```

Messages

The spectrophotometer goes through a multi step calibration sequence. If for any reason the calibration is not completed satisfactorily, the messages displayed, if not self explanatory, are either as follows or relate to a fault condition which requires a service engineer from your local supplier. Other messages are displayed when an accessory is installed.

Error Messages

Display	Possible Causes	Remedy
GLP Calibration Fail		Refer to Appendix GLP Diagnostic Tests
1pt VIS calibrated	Visible region only calibrated	Check and replace UV Lamp if necessary
1pt UV calibrated	UV region only calibrated	Check and replace visible lamp if necessary
Abs Non - Linear (GLP on)	Instrument not at working temperature Misalignment of filter quadrant Dirty Filters	Let instrument reach working temperature Contact service engineer
Too much light	Too much light in sample compartment	Close lid properly and ensure baseplate plug is in place (if relevant)
Beam Blocked !	Not enough light getting to detector	Check light beam is not blocked and cell compartment empty If using a sipper : remove flowcell
Printer Error	RUN PRINT is ON, GLP PRINT OUT is ON, printer is off line or out of paper	Check printer state
No Printer	No active printer	Connect or switch on printer or deselect RUN PRINT and GLP PRINT OUT

Temperature failed	Heated accessory unplugged	Plug in and/or press (sample) (sample)
Call Service E---		Contact service engineer and advise diagnostic error code
Waiting for GLP	Instrument warming up	Refer to Appendix GLP Diagnostic Tests
< Min A	Abs value out of range (<-3.OAU)	Detector seeing too much light. Ensure baseplate plug is fitted Close lid properly
> Max A	Abs value out of range (>+3.OAU)	Sample too concentrated. Something blocking light path.
Vis mirror fail !	Detector did not see enough energy during calibration	Replace the visible lamp

Accessory Messages

After installation of accessory, press (sample) (sample)

4 cell changer	4 position cell changer
6 cell changer	All 6 position cell changers
Single Cell Holder	All single cell holders, except . . . .
Electric Cell	Electric Cell Holder
Thermostat holder	Peltier Cell Holder

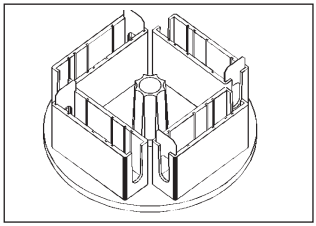
ACCESSORIES

All cell holders accommodate 10 mm pathlength cells as standard, unless stated otherwise. Refer to Essential Safety Notes on page 3.

Multiple Cell Holder Accessories

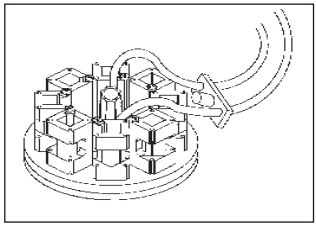
Install by removing existing cell holder, replacing with the new one and turning the central fixing screw until it is finger tight, followed by function Accessory enter

4 position cell changer
80-2106-01



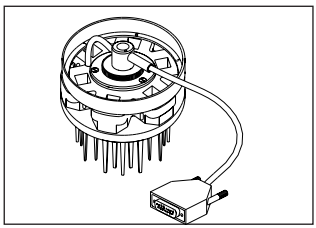
Accessory = 4 cell changer
Select automatic reference
Accommodates cells 10 - 50mm pathlength.

8 position water heated cell changer
80-2109-70



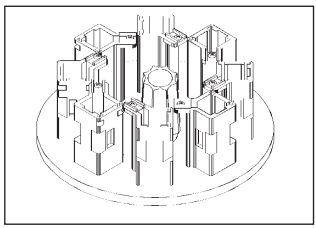
Accessory = 8 cell changer
Select automatic reference
Requires a circulating bath.
Locate round extension of tube restrainer into top of cell changer thumb screw. O rings are to keep tubing together. Replace front blanking plug with that supplied after threading tubes through it.

6 position Peltier heated cell changer
80-2106-04



Accessory = 6 cell changer
Select automatic reference
Requires Temperature Control Unit (80-2105-49).
Cell compartment socket 1

Spare 8 position cell changer
80-2108-01



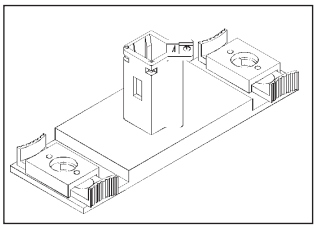
Accessory = 8 cell changer
Select automatic reference

Single Cell Holder Accessories

Install by removing existing cell holder, replacing with the baseplate plug supplied with the accessory, mounting so that the finger locks push backwards and the arrow is on the front face, followed by:

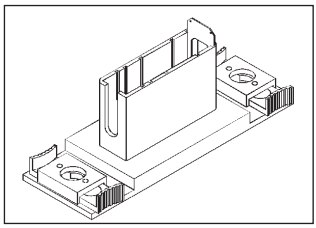
sample sample

Cell Holder, 10mm pathlength
80-2106-05



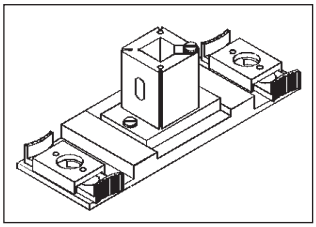
Accessory = Single cell holder

Cell Holder, 50mm pathlength
80-2106-07



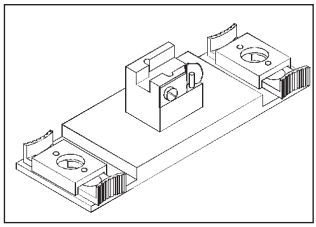
Accessory = Single cell holder

Ultramicrovolume Cell Holder
80-2106-06



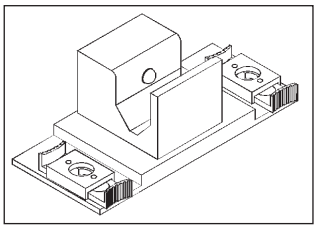
Accessory = Single cell holder
Use with 5µl cell (80-2103-68) and 70µl cell (80-2103-69).
Use 2 axis adjust screws to obtain maximum light throughput

Microvolume Cell Holder
80-2106-09



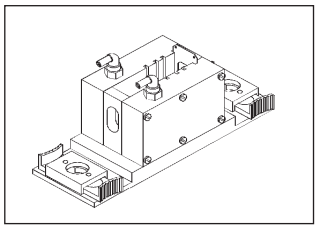
Accessory = Single cell holder
Use with 50µl cell (80-2076-38)

Cylindrical Cell Holder
up to 100mm pathlength
80-2106-10



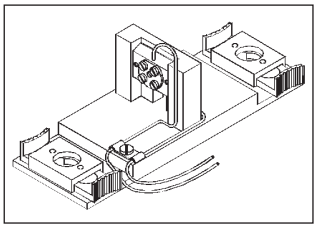
Accessory = Single cell holder

Water Heated Cell Holder
10-40mm pathlength
80-2106-08



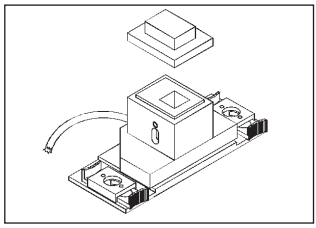
Accessory = Single cell holder
Requires circulation bath. Replace front blanking plug with that supplied after threading tubes through it.

HPLC Cell Holder
80-2106-11



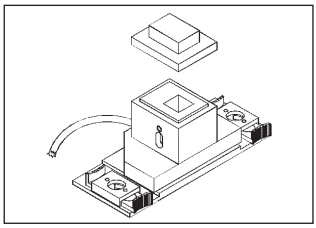
Accessory = Single cell holder
Flowcell volume is 8µl
Pathlength is 2.5mm
Replace front blanking plug with that supplied after leading the two tubes through it.

Peltier Cell Holder
80-2106-13



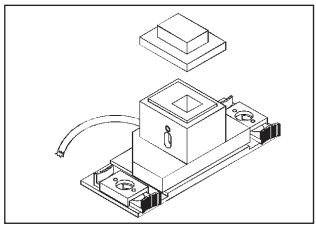
Accessory = Thermostat holder
Set required Temp in range 20-49°C using keypad.
Cell compartment socket 2.

Electrical Cell Holder
80-2106-12



Accessory = Electric cell
Select temperature of OFF, 25, 30, 37°C
Cell compartment socket 2.

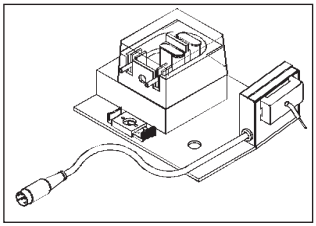
Programmable Heated Cell Holder
80-2106-14



Accessory = Single cell holder
Temp range is 20-100°C
Used for Tm studies of DNA and RNA denaturing. Requires PC and Temperature Control Unit (80-2105-49). Cell compartment socket 1

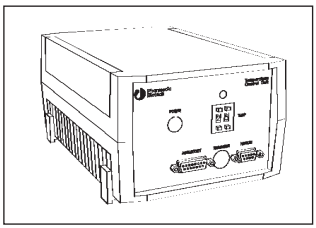
Other Accessories

Sipper
80-2106-15



The Sipper introduces either a sequence of samples for a single reading or a single sample for a series of readings into the spectrophotometer. It is used together with a single 10mm cell holder, either heated or non-heated. A flowcell together with PTFE tubing and pump tubing is supplied with the Sipper. Separate user instructions are supplied with the accessory.

Temperature Control Unit
80-2105-49



This is required to control the 6 position Peltier heated cell changer (80-2106-04) and the Programmable Heated Cell Holder for Tm studies (80-2106-14). Separate user instructions are supplied with the accessory.

Printer Stand	**80-2106-60**
Spare Baseplate blanking plug	**80-2106-18**
Spare dust cover	**80-2106-19**
Cell holder for 100mm pathlength	**80-2107-14**
Cell holder for use with magnetic stirrer (magnetic stirrer required)	**80-2108-10**

Lamps, Consumables and other items

Tungsten halogen lamp	80-2106-16
Deuterium lamp	80-2106-17
Pump head tubes (6) for Sipper	80-2080-74
PTFE flowcell tubing with connectors	80-2055-13
Replacement flowcell (including tubing)	80-2080-60
Autosampler interface kit	80-2104-96
Chart recorder cable	80-2105-95
Recorder REC 101 single channel	18-1001-42
Serial interface cable for connection to PC (D9 male instrument to D9 PC)	80-2105-97
Serial interface cable for connection to PC (D9 male instrument to D25 PC)	80-2106-51
Centronics parallel printer interface cable	80-2071-87

Separate information giving details on serial and parallel interface connections, if required, is available from a Service Engineer with your local supplier, whom you should contact for further details.

SWIFT II Applications Software

SWIFT II comprises application modules for wavelength scanning, reaction kinetics, quantification, multi wavelength, time drive and fraction analysis, and can be used to enhance the software already included on the spectrophotometer. Specific application packages consist of groups of modules for general analytical purposes, biochemistry and molecular biology (SWIFT II - LAB), and for method development and quality control (SWIFT II - METHOD).

80-2108-31 **SWIFT II - LAB - for general analytical purposes**
Wavelength scanning, Reaction Kinetics, Quantification, Time Drive

80-2108-31 **SWIFT II - METHOD - for method development**
Wavelength scanning, Reaction Kinetics, Quantification, Time Drive, Multi Wavelength Fraction Analysis

Recommended PC for proper operation

For optimum performance, an IBM compatible 486 or greater personal computer running Microsoft Windows 95 is required. The PC should have a minimum of 8MB RAM, 200Mb hard disk, a 1.44 MB 3.5 inch floppy disk drive, a serial mouse installed, and free COMMS serial port and VGA graphics. Any printer supported by Microsoft Windows 95 can be used. Contact your supplier for further information.

MAINTENANCE

After sales support - We supply support agreements which help you to fulfil the demands of regulatory guidelines concerning GLP/GMP

- Calibration, certification using filters traceable to international standards
- Certificated engineers and calibrated test equipment
- Approved to ISO 9001 standard

Choice of agreement apart from break down coverage can include

- Preventative maintenance
- Certification

When using calibration standard filters, insert such that the flat surface is facing away from the spring end of the cell holder

Observe all necessary precautions if dealing with hazardous samples or solvents.

User maintenance is restricted to changing the instrument lamps and mains fuse. For any other maintenance operation contact your local supplier.

Lamp Replacement

Replacement lamps are available from your supplier using the following part numbers:

Deuterium lamp	80-2106-17	(includes tungsten lamp as well)
Tungsten lamp	80-2106-16	

The deuterium lamp is supplied fitted onto a plate; a new tungsten lamp is also included.

Although deuterium lamps are covered by their own warranty, an engineer's call out fee is not, and users are advised to change their own lamps. Lamp replacement is very easy, and the process has been designed so that the user can do it without the need to call out a local service engineer. No lamp alignment is required as the lamp select mirror is automatically aligned for maximum lamp energy throughput during the instrument calibration procedure.

The lamps become very hot in use. Ensure they are cool before changing.

Do not touch the optical surfaces of either lamp with your fingers (use tissue); if touched, the area should be cleaned with isopropanol.

To replace a lamp proceed as follows:

1) Switch off the instrument, remove sample from cell holder and disconnect the power supply cord.

2) Locate the lamp access cover on the left hand side of the instrument, push down on the recess and pull the cover off.

3) Undo the fastening screw and remove the cover plate.
4) Slide the lamp plate out and unplug the cable connector.

- If the tungsten lamp has failed, the replacement should be inserted onto the plate, pushing it all the way down into its holder.

- If the deuterium lamp has failed, insert the tungsten lamp onto the plate as above and then replace the whole assembly with the new one.

5) Reconnect the cable connector and slide the lamp plate in until it locates.

6) Replace the black covering plate by re-tightening the fastening screw.

7) Replace the lamp access cover.

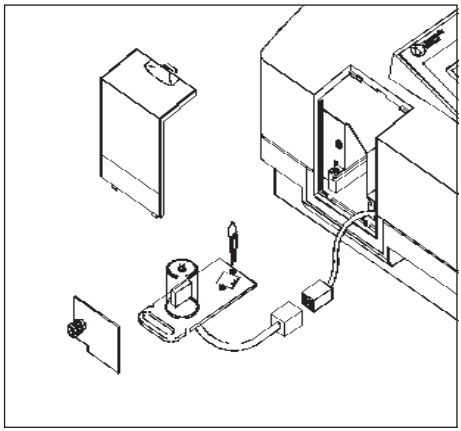
8) Reconnect the power supply cord and switch the instrument on.

9) Reset lamp life to zero and take an original energy reading as appropriate by:

function → Set-up → V → RESET LAMPLIFE → enter

SAVE BASELINE Y/N → > → enter

This is needed for your GLP PRINT OUT.



Deuterium Lamp Warranty

Criteria for lamp replacement are that it must :

- be less that 15 months old AND
- have had less that 750 hours use.

Fuse Replacement

1) Switch off the instrument and disconnect the power supply cord. The fuse holder can only be opened if the power supply plug has been removed, and is located between the power input socket and the on/off switch on the back panel of the instrument.

2) Slide open the fuse holder by pulling at the notch.

3) Place fuses (2A, 5mm x 20mm, FST) into the fuse holder and slide back into position.

4) Reconnect the power supply cord and switch on the instrument.

Fuses are not normally consumed in an instrument's lifetime. If they blow repeatedly contact your supplier.

Cleaning and General Care

External cleaning :

- Switch off the instrument and disconnect the power cord.
- Use a soft damp cloth.
- Clean all external surfaces
- A mild liquid detergent may be used to remove stubborn marks.

Sample compartment spillages :

- Switch off the instrument and disconnect the power cord.
- The cell holders, baseplate and sample compartment are coated in a chemical resistant finish. Strong concentration of sample, however, may affect the surface, and spillages should be dealt with immediately.
- Observe all necessary precautions if dealing with samples or solvents which are hazardous.
- There is a small drain hole in the sample compartment to allow excess liquid to drain away. Liquids will drain onto the bench or table under the spectrophotometer or if preferred, this drain hole can be connected to waste using suitable tubing.
- Remove the cell holder and clean separately.
- Use a soft dry cloth to mop out the sample compartment. Replace the cell holder.
- Reconnect the power cord and switch on the instrument.

APPENDIX

Good Laboratory Practice

Good laboratory Practice (GLP) concerns being able to trace experimental results to an instrument, an operator and the time the result was obtained so that a laboratory can prove that the instrument was functioning correctly or not. Laboratory, operator and internal instrument reference names can be entered on the spectrophotometer

On calibration or re-calibration, the instrument checks the parameters and presents the status for the customer's acceptance prior to going into normal operation. If GLP PRINT OUT is ON, a print out of the results of these tests is then done. The date, time and operation are printed when parameters are printed. GLP requires that the readings underlying any result shall be recorded, and GLP PRINT OUT is ON performs this task automatically and prints the results.

GLP Diagnostic Tests

The integrity of the instrument for GLP purposes is quantified from :-

- the calibration status of the instrument
- the age and % energy of the lamps compared to their values when new
- the wavelength accuracy by comparing to the 656nm deuterium line
- the values of a built in absorbance filter compared to when the instrument was manufactured (or last serviced by an accredited engineer).
- the bandwidth at 656nm
- the instrumental stray light

The expected values are given in parentheses on the GLP print out after calibration, and the range of acceptance is defined in the technical specification of the instrument.

In the unlikely event that the instrument fails calibration or goes out of specification, a sequence of error messages will appear on the display, the final message being "GLP CALIBRATION FAIL". These should be acknowledged sequentially by pressing **enter**, and checking them with reference to the MESSAGES section of the user manual. If a parallel printer is connected, doing so will provide further information on the GLP print out.

The following should be checked :-

- is the cell compartment lid closed properly
- is a sample in the light beam - if so, remove it
- is the baseplate plug in place (single cell accessory)
- is the blanking plug at the front of the cell compartment in place

Pressing **enter** after the message "GLP CALIBRATION FAIL" appears on the display confirms that you have accepted the instrument status. This status does not necessarily mean that the instrument cannot do the measurement required by the protocol for your experiment, but having looked at the GLP print out you may wish to contact your local service engineer for advice.

During calibration, if "GLP PRINT OUT" is on, the instrument will wait 10 minutes to allow working temperatures to be reached before completing calibration. The display will show "Waiting for GLP"; press **stop** to abort this if required.

Entry of alphanumeric characters for print out

function > Parameters V LAB NAME enter

OPERATOR

INSTRUMENT

When using the above utilities, the nature of the keyboard keys changes.

A title (40 characters maximum) made from an alphanumeric character string of upper and lower case characters, numbers and punctuation marks may be entered.

abcdefghijklmnopqrstuvwxyz = space
,.; 1234567890-=/ \
ABCDEFGHIJKLMNOPQRSTUVWXYZ
<>: !@#'%^&*()_+? |

An individual character is chosen using the ^ V keys and selected using the **PRINT** key. The > key moves the cursor along the display. A complete name or title is entered using the **ENTER** key.

< > Move cursor along the display

^ V Move within the character string

mode V Move to start of character string ("a")

wave V Move $^1/_4$ way down character string to "x"

set ref V Move $^1/_2$ way down character string to "A"

sample V Move $^3/_4$ way down character string to "X"

function Delete the character at the cursor

print Select the character at the cursor

enter Complete the alphanumeric entry

GLP print out at instrument calibration (GLP PRINT OUT on) :

Example :

```
Laboratory  name  ..................                    #

Instrument Ref ..................                     #

 Ultrospec 2000 UV/Vis Spectrophotometer
Serial no     : 63035
Software      :  4090  V2.0  19951115
              : Slave V1.7
Last serviced : 19960219

Instrument state at calibration
Calibrated 19960219 at 15:12
Calibration 2 point  valid 190 - 1100nm

Bandwidth at 656nm  (3nm) : 2.6nm :      PASS

Wavelength (656nm) : 656.0nm :           PASS

Absorbance  expected  :  read
at  220nm   (2.414)  :  2.417  :     PASS
at  340nm   (1.986)  :  1.992  :     PASS
at  500nm   (1.488)  :  1.489  :     PASS

Stray light at220nm ( <0.025%T):
                      0.012%T :          PASS

UV Lamp :  101% of original energy                   *
Vis lamp : 100% of original energy                   *

Current instrument state

Accessory : six position cell changer
There are no reference readings made by the
cell changer

UV lamp... installed 19960219 at 10.03
total use 5 hours
baseline in use dated      19960219 at 15:01
stored baseline date       19960219 at 15:01

Vis lamp... installed      19960320 at 10:03
total use 41 hours
baseline in use dated      19960219 at 15:02
stored baseline date       19960219 at 15:02
```

The GLP and header print outs are obtained after instrument calibration, and assumes a printer is connected and the GLP Print Out option is ON. The instrument waits 10 minutes to allow the lamps to warm up properly.

\# These are printed out for you to complete manually. See earlier if you wish to enter your details for printing out.

* % of power of lamp measured at calibration relative to when the lamp life was last set to zero.

SPECIFICATION

Wavelength range	190 - 1100nm
Monochromator	1200 lines/mmAberration corrected concave grating
Spectral bandwidth	3nm
Wavelength accuracy	± 1nm
Wavelength reproducibility	± 0.5nm
Light sources	tungsten halogen and deuterium lamps
Detector	silicon photodiode
Photometric range	- 3.000 to 3.000A, 0.01 to 99999 concentration units, 0.1 to 200%T
Photometric accuracy	± 0.5% or ± 0.003A to 3.000A, whichever is the larger
Photometric reproducibility	within 0.5% of absorbance value
Stability	± 0.001A per hour at 340nm (tungsten lamp)
Stray light	<0.025 %T at 220nm using NaI and <0.025 %T at 340nm using $NaNO_2$
Analogue output	100mV per 1.000A
Digital output	9 pin serial and Centronics parallel
Sample compartment size	210 x 140 x 80mm
Dimensions	500 x 360 x 190mm
Weight	13kg
Power requirements	90-265 V AC , ± 10%, 50/60Hz, 150VA
Safety Standard	EN61010-1
EMC emissions	EN 50 081-1 Generic emissions part 1
EMC immunity	EN 50 082-1 Generic immunity part 1
Mains harmonies	EN 61000-3-2
Susceptibility Standard	IEC801
Quality System	Designed and manufactured in accordance with an ISO9001 approved quality system

Specifications are measured after the instrument has warmed up at a constant ambient temperature and are typical of a production unit.
As part of our policy of continuous development we reserve the right to alter specifications without notice.

Warranty

Your Supplier guarantees that the product supplied has been thoroughly tested to ensure that it meets its published specification. The warranty included in the conditions of supply is valid for 12 months only if the product has been used according to the instructions supplied.
Your Supplier can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of this product.
Biochrom Ltd, 22 Cambridge Science Park, Milton Road, Cambridge CB4 0FJ, England.

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